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NEW & NOTEWORTHY This study has identified that sympathetically mediated blood pressure regulation is reduced following ascent to high altitude. Additionally, we show that high altitude Andean natives have reduced blood pressure responsiveness to SNA outflow compared to Nepalese Sherpa. However, basal sympathetic activity is inversely related to the magnitude of SNA-mediated fluctuations in blood pressure regardless of population or condition. These data set a foundation to explore more precise mechanisms of blood pressure control under conditions of persistent sympathetic activation and hypoxia.

ABSTRACT:

High-altitude (>2500m) exposure results in increased muscle sympathetic nervous activity (MSNA) in acclimatizing lowlanders. However, little is known about how altitude affects MSNA in indigenous high-altitude populations. Additionally, the relationship between MSNA and blood pressure regulation (i.e., neurovascular transduction) at high-altitude is unclear. We sought to determine 1) how high-altitude effects neuro-cardiovascular transduction and 2) whether differences exist in neuro-cardiovascular transduction between low and high-altitude populations. Measurements of MSNA (microneurography), mean arterial blood pressure (MAP; finger photoplethysmography), and heart rate (electrocardiogram) were collected in: I) lowlanders (n=14) at low (344m) and high-altitude (5050m), II) Sherpa highlanders (n=8; 5050m), and III) Andean (with and without excessive erythrocytosis) highlanders (n=15; 4300m). Cardiovascular responses to MSNA burst sequences (i.e. singlet, couplet, triplet, and quadruplets) were quantified using custom software (coded in MATLAB, v2015b). Slopes were generated for each individual based on peak responses and normalized total MSNA. High altitude reduced neuro-cardiovascular transduction in lowlanders (MAP slope: high-altitude, 0.0075±0.0060 vs low-altitude, 0.0134±0.080; p=0.03). Transduction was elevated in Sherpa (MAP slope, 0.012±0.007) compared to Andeans (0.003±0.002; p=0.001). MAP transduction was not statistically different between acclimatizing lowlanders and Sherpa (MAP slope, p=0.08) or Andeans (MAP slope, p=0.07). When accounting for resting MSNA (ANCOVA), transduction was inversely related to basal MSNA (bursts/min) independent of population (RRI, r= 0.578 p<0.001; MAP, r= -0.627 p<0.0001). Our results demonstrate transduction is blunted in individuals with higher basal MSNA, suggesting blunted neuro-cardiovascular transduction is a physiological adaptation to elevated MSNA rather than an effect or adaptation specific to chronic hypoxic exposure.

INTRODUCTION:

Sympathetic nervous system activity (SNA) has been shown to increase following exposure to high altitude in lowlanders (12, 17, 22, 26, 28, 39). The mechanism(s) governing persistent sympatho-excitation during hypoxic exposure remain unclear, but have previously been attributed to an increase in peripheral chemoreceptor drive (26, 31), elevated intracranial pressure (27), increased pulmonary artery pressure (29), or a combination of these factors. While heightened sympathetic outflow at altitude signals for global vascular constriction, mean arterial pressure (MAP) and total peripheral resistance (TPR) are maintained near sea level values during acclimatization (4, 28). This shift in communication between the nervous system and the vasculature (i.e., neurovascular transduction), indicates that there may be a reduction in the efficacy of SNA to effect vascular tone under conditions of prolonged hypoxia. The causes and consequences of this uncoupling remain poorly understood.

Of further interest are populations indigenous to high altitude, such as the Tibetan (Sherpa) and Peruvian (Andean) highlanders, who have been exposed to hypobaric hypoxia for millennia. Although there is considerable debate regarding specific durations at altitude, it is generally accepted that the Old World Plateaux (Ethiopian and Tibetan) have been settled for longer than the Altiplano in the New World (Andes) (1–3, 25, 43). This is suggestive that duration at altitude may play a role in the respective patterns of adaptation between high altitude populations. We have previously observed that Tibetan Sherpa show an overall lower degree of sympathetic activation compared to acclimatizing lowlanders, while having similar resting arterial pressure and similar or lower reactivity to heightened sympathetic stress (7, 28). In contrast, high altitude populations in the Andes exhibit a high reported incidence of excessive erythrocytosis (EE; defined as having a [Hb] >21g/dL in males, >19g/dL in females), which has been linked with vascular dysfunction and increased risk of cardiovascular disease (21, 35, 38). Interestingly, EE is extremely rare in Tibetan high altitude natives (38), suggesting distinct differences in the patterns of adaptation between these two high altitude populations.

Differential adaption to long term hypoxic exposure between these two groups necessitates further investigation into mechanisms of cardiovascular control.

Although SNA and arterial pressure has been previously documented at altitude in both low and high altitude populations (3, 5, 7, 17, 22, 28), there is limited work that has investigated the transduction of sympathetic outflow to the integrated control of blood pressure in response to hypoxic exposure. Furthermore, there are no studies that have attempted to identify whether population-based differences exist in this aspect of sympathetic control. Thus, we aimed to characterize the relationship between spontaneous fluctuations in SNA to cardiovascular responses in North American and European lowlanders, Tibetan Sherpa, and Peruvian Andeans (both with EE+ and without EE). While renal adaptation and a shift in blood volume at high altitude also contribute to the integrated control of blood pressure, this study focuses on neuro-cardiovascular mechanisms. To address this, we analyzed muscle sympathetic nervous activity (MSNA) and simultaneous hemodynamic data collected during two previous high altitude research expeditions (Nepal 2016, and Peru 2018) to assess the impact of SNA on blood pressure regulation at rest. Our hypotheses were twofold: first, we hypothesized that lowlanders would show a blunted neuro-cardiovascular transduction response at high altitude compared to sea level. Second, we hypothesized that Sherpa would show a greater neuro-cardiovascular transduction response compared Andeans and acclimatizing lowlanders, due to previously observed (7, 28) lower reactivity to sympathetic stress.

METHODS:

Data for the current investigation was collected over two previous research expeditions to Nepal (UBC-Nepal Expedition; (42) and Peru (Global REACH 2018; (36). We have previously published on basal MSNA (28), and reactivity to exercise and breath-holding in the Sherpa (7). However, the analyses performed as part of this investigation address a specific novel hypothesis and report data not previously published. Specifically, the current study focuses on novel analyses related to the transduction of spontaneous fluctuations in SNA to mean arterial pressure (MAP) and heart rate (R-R Interval) responses over a ~10 minute baseline period. Although participants took part in a number of independent investigations during the above mentioned expeditions, care was taken to ensure that there was no overlap between studies, and each study addressed distinct *a priori* research questions.

Study Participants

Participants were comprised of lowlanders (n=14; 27±1yrs; 2 female), Nepalese Sherpa (n=8; 32±5yrs) and Peruvian Andeans (n=15; 42±3yrs). Note, the Andean group included 7 healthy (i.e. non-EE) and 8 EE men. Data were grouped for the healthy and EE Andeans since no differences in neuro-cardiovascular transduction were observed (details below). While demographics, resting hemodynamic function and basal metrics of MSNA have been published previously, these values are reported in Table 1 for completeness and context. Lowlanders, Sherpa, and Andean participants were free of any known respiratory, cardiovascular, metabolic, and neurological disorders as determined by a self-reported health history questionnaire. No participants were taking any medication at the time of testing. Lowlander participants were members of a larger expedition to Nepal in 2016 (42), and the Sherpa highlanders were recruited during the same expedition from the Khumbu Valley in Nepal. Pre-expedition testing of lowlanders was performed at 344 m (Kelowna, Canada), and then traveled to Nepal and ascended over 9-10 days to 5050m. Sherpa were not on any medication and were tested on

days 1-3 following arrival at 5050m, while Lowlanders were tested between days 1-10. Refer to Willie et al 2018 (39) for a more detailed description of ascent profiles.

Andean participants were recruited and tested as part of a second expedition (Global REACH 2018) (34) to Cerro de Pasco, Peru in 2018 (4300 m). Andeans with EE were diagnosed prior to being contacted and recruited using an existing local database (venous [Hb] concentration 22.5±0.91 g/dL). All Andeans were born above 3250m and were permanent residents of Cerro de Pasco.

High altitude residents provided informed written consent in their native language, with procedures explained in the local dialect as needed. Local Ethical approval was obtained for both expeditions by the University of Alberta Biomedical Research Ethics Board (Pro00064195 and Pro00077330), Nepal Health Research Council, and Universidad Peruana Cayetano Heredia (#101686)

Data Collection

All participants were tested in the supine position. All data were recorded and synced using Labchart (ADInstruments, Chart Pro v8.3.1, Australia). Heart rate (Electrocardiogram lead II), and the non-invasive arterial blood pressure waveforms (finger photoplethysmography; Finometer Pro, Finapres Medical Systems, Netherlands) were collected continuously at 1 KHz (ADInstruments, Chart Pro v8.3.1, Australia). Heart rate (HR) was calculated from the ECG R-R interval. Beat-by-beat mean arterial pressure (MAP), systolic (SBP) and diastolic (DBP) pressures were calculated from the arterial pressure waveform that was calibrated against manual sphygmomanometry (averaged from three separate readings) during rest. Beat-by-beat cardiac output (CO) was also calculated using the Model Flow algorithm and used to calculate total peripheral resistance (TPR = MAP/CO) and conductance (TPC = CO/MAP). Microneurography was used to directly measure muscle sympathetic nerve activity (MSNA). A tungsten microelectrode (200μm diameter, 35 mm long, tapered to a 1-5 μm uninsulated tip)

was inserted percutaneous into the peroneal (common fibular) nerve, with an additional uncoated tungsten reference electrode inserted subcutaneously 1-3 cm from the recording site. The recording electrode was manipulated until a pulse-synchronous bursting pattern was identifiable in response to apnea but not a loud noise (16). The raw MSNA signal was acquired (Neuroamp EX headstage, ADInstruments; model 662C-3, Iowa University Bioengineering, USA), amplified (1000x pre-amplifier and 100x variable gain isolated amplifier), band pass filtered (700-2,000Hz), rectified, and integrated (decay constant 0.1s) to obtain a mean voltage neurogram. The Neuroamp was used to collect MSNA data during the 2018 Global REACH expedition; the model 662C-3 was used for MSNA data collection during the 2016 UBC-Nepal Expedition (at both low and high altitude). Both raw and integrated signals were sampled at 10 KHz (ADInstruments, Chart Pro v8.3.1; Australia).

Data Analysis

MSNA bursts were identified using a semi-automated detection algorithm (Chart Pro 8.3.1) and confirmed by a trained observer (SAB/CDS) based on a pulse-synchronous pattern observed from both raw and integrated MSNA neurograms. Baseline MSNA was quantified as burst frequency (bursts/min) and incidence (bursts/100 heart beats). MSNA, peripheral oxygen satruation, and other cardiovascular metrics were extracted on a beat-by-beat basis for each individual over 11 ± 5 minutes during baseline conditions at low altitude (lowlanders; 334m) and high altitude (lowlanders; 5050m, Sherpa; 5050m, Andeans; 4300m).

MSNA and hemodynamic variables for each individual were saved to Excel spreadsheets and read into custom software written in MATLAB (MATLAB 2015b; The MathWorks, Natick, Massachusetts) (32) to quantify the effect of neuro-cardiovascular transduction on measured hemodynamic parameters. The software identified MSNA burst locations via LabChart comment markers. Once identified, bursts were aligned with respect to the beat-by-beat data. Once aligned, MSNA was filtered to determine the position of all

recorded burst sequences consisting of single or consecutive groups of bursts separated on each side by 1 cardiac cycle without MSNA. Sequences consisting of singlet, couplet, triplet, or quadruplet (4 or more) bursts were grouped together for analysis (as per Steinback et al 2019) (32). Following the last burst in each sequence, the change in blood pressure, R-R interval and associated Finometer derived changes in cardiac output were tracked over the subsequent 15 cardiac cycles, similar to the method described in previous studies (14, 15, 32). MAP, R-R interval and cardiac output data were used for analysis in order to comprehensively characterize systemic transduction. The mean change in MAP, R-R interval and cardiac output for different sequences was calculated by the software for each participant and saved to spreadsheets along with the standard deviation and number of burst sequences recorded. Peak changes in MAP, R-R interval and cardiac output were identified for each sequence type were subsequently grouped and overlaid to obtain a mean transduction for each participant (Figure 1) (32). Sequences of "non-bursts" were analyzed in a similar manner, with MAP, R-R interval and cardiac output indexed to sequences of cardiac cycles without bursts. To identify whether changes in R-R interval and cardiac output were directly associated with changes in MAP, we conducted a time to peak analysis for measures of MAP, R-R interval, and cardiac output.

Additionally, bursts were grouped into a quartile range (Q1-Q4) within each sequence, with Q1 representing the smallest summed amplitude of bursts, and Q4 being the largest summed amplitude within a given sequence. To account for individual differences in mean burst amplitude, burst amplitude was normalized to the mean summed amplitude within singlet Q1 sequences (SQ1) which was set to 100% for each individual. All subsequent amplitudes for all quartiles were calculated as a percentage of SQ1. This normalization allowed for the comparison of quartile data between subjects and across groups. A mean transduction response for each individual was calculated as the slope of the peak responses in outcome plotted against the 16 normalized burst amplitude quartiles (i.e. 4 sequence types x 4 amplitude quartiles). Slopes were weighted (IBM SPSS statistics 25, United States, 2017) to account for

the number of occurrences (proportion) of quartiles within each sequence. Two Andean participants were excluded from the quartile analysis due to lack of data (<6 data points as opposed to 16; 4 sequences x 4 quartiles). The relationship between total normalized burst amplitude (per quartile) and physiological outcome (peak physiological response) is depicted in Figure 1. Individual slopes were then used to obtain a mean transduction response per group. As an additional analysis, we assessed the relationship between previously reported baroreflex gain data (28, 30) and the generated transduction slope.

Statistical Analyses

Comparisons were made between 1) lowlanders at low- and high-altitude, and 2) Sherpa, Andeans, and acclimatizing lowlanders. The dynamic relationship of transduction across cardiac cycles was compared within a given group using one-way ANOVAs. Holm-Sidak post-hoc analyses were conducted where main effect of group was identified. To assess the influence of changes in R-R interval on the MAP response, ANCOVA analyses were used, incorporating R-R interval as covariate. Between-group comparisons for lowlanders from low -to high-altitude were assessed using pre-planned contrasts (paired T-tests), with an adjusted alpha (α') value corrected for multiple comparisons (c). This was performed by adjusting the a priori alpha (α, 0.05) using the experiment-wise error rate (α_e) (6, 18):

$$a' = \frac{a_c}{c}$$
$$a_c = 1 - (1 - a)^c$$

Alpha for comparisons between lowlanders at low and high altitude was corrected to p<0.046.

Relationships between variables were evaluated using Pearson correlations and linear regression. To account for effect of resting MSNA on mean transduction responses, an ANCOVA analysis was run incorporating basal burst frequency as a covariate. Data are expressed as mean ± standard deviation (SD) unless otherwise indicated. All statistical

263 analyses performed using SigmaStat v14.0 (Systat Software). A p-value of <0.05 was
264 considered statistically significant.
265
266

RESULTS

Participant demographics and baseline metrics. Participant demographics, baseline cardiovascular and MSNA metrics are reported in Table 1. Although lowlander and Tibetan Sherpa data have been reported elsewhere (6, 28), they are included in this novel analysis to enable comparison with Peruvian Andeans.

Andeans were older compared to lowlanders (p=0.001) but not different to Sherpa. (p=0.065). Andeans also had significantly elevated body mass index (kg/m^2; BMI) compared to lowlanders and Sherpa (p=0.002 and p=0.027, respectively). There were no significant differences in SBP, DBP or MAP in lowlanders at high and low altitude (p=0.07, p=0.15, p=0.26, respectively). There were no significant differences between high altitude groups in SBP, DBP or MAP (main effects of p=0.871, p=0.154, p=0.773, respectively). Resting MAP was not significantly different between EE and non-EE Andeans (85±2 vs 89±8 mmHg, respectively; p=0.24). Although Andean participants without EE appeared to have elevated burst frequency compared to Andeans with EE (44±14 vs 37±11 bursts/min, respectively), these differences were not statistically significant (unpaired t-test; p=0.36). Additionally, the mean transduction slopes for both MAP and RRI were not different between EE and non-EE Andean groups (p=0.35 and p=0.60, respectively). Therefore, EE and non-EE participants were grouped together as Andeans for the subsequent analyses.

Representative burst patterns for all groups are shown in Figures 2. At high altitude, lowlanders had significantly elevated MSNA burst incidence and frequency (p<0.001) compared to low altitude. Despite Sherpa being tested at a higher altitude than Andeans (5050m vs 4330m), Sherpa had significantly lower burst frequency than Andeans (p=0.006), as well as lower burst incidence compared to both lowlanders and Andeans (p=0.036 and p=0.001, respectively). Elevated burst frequency was associated with a lower proportion of single burst sequences and an elevated proportion of multiple burst sequences in both lowlanders at high altitude and Andeans (Figure 3). Conversely, lower burst frequency corresponded with a higher

proportion of singlet sequences and lower proportion of multiple burst sequences in lowlanders at low altitude and Sherpa (Figure 3).

The impact of high altitude on neuro-cardiovascular transduction in lowlanders

Cardiac and pressor response to burst sequences. Changes in MAP following MSNA sequences were greater at low compared to high altitude in lowlanders for both singlet (2.2 ±1.1 vs 0.83 ±0.58 mmHg; $p<0.001$) and couplet (4.2±2.0 vs 1.8±1.6 mmHg; $p=0.005$) sequences, but not different for triplet and quadruplet sequences ($p=0.699$; $p=0.547$, respectively). However, the nadir in MAP following triplet and quad non-burst sequences was greater in lowlanders at high altitude ($p=0.041$ and $p=0.001$; Figure 4).

Following SNA bursts, there was an acute cardio-acceleration (decrease in R-R interval) occurring within 5 cardiac cycles. There were no significant differences between lowlanders at low and high altitude in decrease in R-R Interval across any burst sequence (singlets, $p=0.575$; couplets, $p=0.69$; triplets, $p=0.56$; quad, $p=0.30$). Following non-burst sequences, there was an acute cardio-deceleration (increase in RR interval). There were no differences between lowlanders at low versus high altitude in the R-R interval response to non-burst sequences (Figure 5).

Mean Transduction Slope. Mean transduction slope was greater in lowlanders at low altitude for R-R interval (low altitude, 0.00017 ± 0.00014; high altitude, 0.00007 ± 0.00008; $p=0.032$). The transduction slope for MAP was blunted at high altitude (MAP slope, 0.0075±0.0060 at high altitude versus 0.0134 ±0.0080 at low altitude; $p=0.03$). To examine the influence of altered basal SNA on transduction, an ANCOVA was run including resting MSNA (burst frequency) as a covariate. This analysis subsequently indicated no difference between low and high altitude for R-R interval or MAP slopes ($p=0.718$ and $p=0.278$; ANCOVA).

Differences in neuro-cardiovascular transduction between Sherpa, Andeans, and acclimatizing lowlanders

Cardiac and pressor response to burst sequences. Following bursts of MSNA, Sherpa showed a greater MAP response compared to Andeans ($p<0.001$) and lowlanders at high altitude ($p=0.010$) across all sequence types (main effect of group, $p<0.001$; Figure 4). Acclimatizing lowlanders showed a greater MAP response to MSNA bursts compared to Andeans ($p<0.001$). The drop in MAP (nadir) following non-burst sequences was greatest in lowlanders at high altitude (main effect of group, $p=0.017$; Figure 4). Subsequent post hoc analyses indicated that the overall fall in pressure following non-burst sequences appeared greater, but was not statistically different between lowlanders and Sherpa ($p=0.152$) but was greater in lowlanders compared to Andeans ($p=0.017$) (Figure 4).

The decrease in R-R interval following burst sequences was different between groups (main effect of group, $p<0.001$; Figure 5). Sherpa showed a greater drop in R-R interval compared to Andeans ($p=0.003$) but this was not statistically different to lowlanders ($p=0.051$). However, lowlanders exhibited a greater decrease in R-R interval compared to Andeans ($p<0.001$) across all burst sequences. The cardio-deceleration (decrease in R-R interval) was not difference between groups following non-burst sequences ($p=0.129$) (Figure 5).

Mean Transduction Slope. Using quartiles data, a main effect of group was identified for both MAP and R-R interval mean transduction slopes ($p=0.04$; $p=0.006$, respectively; Figure 6 & 7). Sherpa had a significantly greater slope for both MAP (0.012 ± 0.007) and R-R interval compared to Andeans (MAP, 0.0031 ± 0.0024; R-R Interval, 0.00003 ± 0.00004) ($p=0.003$ and $p=0.005$) and a greater R-R interval slope compared to lowlanders ($p=0.046$). While Sherpa tended to have a greater MAP transduction slope compared to lowlanders this was not statistically significant ($p=0.08$). Lowlanders were not different from Andeans for R-R interval slope ($p=0.201$) or MAP slope ($p=0.07$).

The peak transduction in MAP was inversely related to prevailing degree of sympathetic activity, independent of group ($r= -0.627$, $p<0.001$; Figure 6). Peak transduction in R-R Interval was also inversely related to prevailing sympathetic activity independent of group ($r=0.578$;

p<0.001). Subsequent analysis indicated that mean transduction slopes were no longer significantly different between groups for either MAP or R-R interval slopes (p=0.160; p=0.203, respectively) when concurrent SNA burst frequency was taken into account as a covariate (ANCOVA).

Time to peak responses

Time to peak in lowlanders. Time to peak was assessed in all groups for cardiac output (CO), MAP, and R-R Interval. At low altitude, changes in MAP occurred 5.6 ± 1.9s following burst sequences; changes in R-R Interval occurred 2.1 ± 1.0s following bursts; changes in CO occurred 2.4 ± 1.0s following bursts. Peak MAP responses followed both peak R-R interval (p<0.001) and peak changes in CO (p<0.001). Time to peak in R-R Interval and CO were similar (p=0.46). At high altitude, peak changes in MAP (4 ± 2s) also followed peak changes in both R-R Interval (1.9 ± 1.1s) and CO (2.9 ± 1.1s) (p<0.001). Again, time to peak changes in R-R Interval and CO were similar (p=0.23).

Time to peak in Sherpa and Andeans. In Sherpa, time to peak change in MAP (4.6 ± 2.5s) followed peak changes in both CO (2.4 ± 1.3s) and R-R interval (2.1 ± 1.5) (p<0.001). Time to peak for CO and R-R interval were similar (p=0.56). In Andeans, time to peak changes in MAP (3.1 ± 2.5s) also followed peak changes in CO (1.8 ± 1.0s) and R-R interval (1.3 ± 1.0) (p<0.01). Time to peak was similar between CO and R-R interval (p=0.17).

Discussion

The purpose of the current investigation was to characterize the transduction of spontaneous bursts of MSNA to cardiovascular outcomes in low and high-altitude populations. The main findings were: 1) lowlanders exhibited a blunted neuro-cardiovascular transduction slope at high altitude compared to sea level, but had a greater drop in blood pressure during non-burst sequences; 2) Tibetan Sherpa showed an elevated transduction response compared to Andeans, who had consistently blunted neuro-cardiovascular transduction; 3) the increase in blood pressure following bursts of sympathetic activity was inversely related to prevailing levels of MSNA independent of population (ANCOVA, $p<0.001$). Andeans (EE+ and EE-), who had the highest resting values for burst frequency and incidence, consistently demonstrated a blunted pressure (MAP) and heart rate (R-R interval) response to burst sequences compared to other groups (Figures 4 & 5). Conversely, Sherpa showed greater vascular (MAP) and cardiac (R-R interval) responses to burst sequences despite significantly lower resting SNA, indicating an elevated transduction (Figures 4 & 5). These findings imply that neuro-cardiovascular transduction is an inverse function of resting sympathetic activity, and thus may be representative of a broader physiological adaptation to maintain normotensive pressure. Alternatively, alterations (elevation or decrease) in the level of sympathetic signaling may be required to compensate for blunted or heightened vascular responsiveness to vasoconstrictor signals. Further interventional studies are required to delineate these mechanisms.

The impact of high altitude on neuro-cardiovascular transduction in lowlanders

Our findings demonstrate that transduction was blunted in lowlanders at high altitude. This may be attributable to specific physiological changes which occur in response to hypoxic exposure. Exposure to acute hypoxia is associated with a vasodilatory response (9, 23) which may be balanced by the concomitant sympatho-excitatory response (20). The dilatory influence of hypoxia may be reflected as the greater fall in pressure in non-burst sequences. The current

findings demonstrate that the nadir pressor response (MAP) following non-burst sequences was greater in lowlanders at high altitude (specifically for triplets and quadruplets+; Figure 4), supporting the idea that an opposing dilatory response offsets the vasoconstrictor effects of sympathetic activity following sympathetic bursts. This is supported by previous literature documenting the influence of vasodilatory pathways on vascular function in lowlanders (9), and concurs with the notion that the increase in sympathetic nervous system in response to hypoxia may mask a greater hypoxic vasodilation (23). Additionally, it could be interpreted that the elevation in SNA in acclimatizing lowlanders occurs to offset the influence of hypoxia mediated vasodilation and hence act to preserve arterial pressure. Furthermore, a shift to a greater proportion of larger burst sequences (Figure 3) may be beneficial in offsetting hypoxic vasodilation. Maintaining oxygen delivery in conditions of hypoxic stress while modulating sympathetic outflow to defend against hypotension represents a complex homeostatic interaction in the control of blood pressure.

Differences in neuro-cardiovascular transduction between Sherpa, Andeans, and acclimatizing lowlanders

Our results indicate that neuro-cardiovascular transduction is greatest in Sherpa whereas transduction was overall blunted in the Andeans, with acclimatizing lowlanders falling in the middle of these two populations. Despite differences in resting MSNA between groups, (with Sherpa exhibiting low activity and Andeans exhibiting highest levels of activity), all groups displayed similar values for blood pressure. Taken together, these findings indicate that the inverse relationship between neuro-cardiovascular transduction and sympathetic activity is likely an adaptive mechanism to maintain normal blood pressure. Previous work in patients with obstructive sleep apnea showed that higher resting sympathetic outflow in the absence of blunted transduction resulted in an elevation in blood pressure, suggesting that unaltered vascular transduction may contribute to the development of hypertension (33). Conversely, we

have demonstrated a blunted transduction during healthy pregnancies that appears to offset sympathetic hyperactivity and maintain blood pressure (32). This supports our interpretation that an adaptive resetting of neuro-cardiovascular transduction is an important response to maintain normotensive pressure.

It has been previously reported that transduction is inversely related to sympathetic baroreflex gain in young males (19).We have previously shown that there is an upward resetting of the baroreflex upon ascent to high altitude, while Sherpa appear to have a lower baroreflex operating point compared to acclimatizing lowlanders (28). Additionally, baroreflex operating point has been reported to be similar between EE+ and EE- Andeans (30). Considering the interaction between blood pressure control of SNA (baroreflex) and SNA control of blood pressure (transduction), it is possible that baroreflex sensitivity may be an important regulatory factor in the capacity of the cardiovascular system to buffer fluctuations in SNA. However, in a subsequent analysis of our data we did not observe a relationship between previously reported baroreflex gain values (28, 30) and transduction slope in the groups studied (lowlanders 344m, r= -0.02; lowlanders 5050m, r= -0.2; Sherpa, r= 0.7; Andeans, r= -0.2). The incongruity between our findings and those previously published by Hissen et al (2019) may arise due to differences in methodological quantification of transduction. However, based on our current analyses, sympathetic baroreflex gain does not appear to be related to the transduction response.

While renal adaptation and shift in blood volume over time at altitude may contribute to control of blood pressure, our analyses focus specifically on acute neuro-cardiovascular control. The mechanism(s) by which transduction is altered between high altitude groups remains unclear. There may be a change in alpha adrenergic sensitivity or density to account for level of MSNA in order to mitigate the magnitude of changes in pressure. Under resting conditions, tonic sympathetic control over vascular tone is mediated primarily by noradrenaline binding to alpha 1 and 2 adrenergic receptors (10, 13). Changes in this distribution (i.e., changes in sensitivity or receptor density) over time at high altitude may account for differences in neuro-cardiovascular

transduction. This is supported by studies documenting a blunted vasoconstrictor response to direct adrenergic stimulation in conscious rats following 4 weeks of hypoxic exposure (11, 24). Although vascular sensitivity was not assessed in the current study, it has been previously reported that healthy individuals at sea-level with higher resting MSNA demonstrate lower vascular responsiveness to adrenergic stimulation, indicating that there is an offsetting of MSNA at the level of the vasculature (8). Reduction in vascular sensitivity to MSNA could explain blunted transduction in Andeans, who exhibited the highest resting sympathetic activity despite having similar blood pressure to other groups. Reduced vascular sensitivity may be a physiological adaptation to higher resting sympathetic outflow. Alternatively, it could be interpreted that sympathetic outflow increases to account for low vascular sensitivity. Additionally, differences in noradrenaline release, uptake, or degradation at the level of the nerve terminal could contribute to the observed differences in the blood pressure response to sympathetic outflow. The precise mechanisms underlying blunted (or elevated) transduction have yet to be explored.

While our results indicate that the blunting (or elevation) in neuro-cardiovascular transduction is related to prevailing sympathetic activity, phenotypic differences may in turn drive the variation in resting sympathetic activity. Sherpa have been previously characterized as having lower basal sympathetic activity but a greater vascular responsiveness to sympathetic vasomotor drive compared to acclimatizing lowlanders (28). Thus, elevated transduction may be a physiological mechanism acting in concert with lower resting sympathetic activity to maintain vascular tone. Conversely, certain high altitude Andean populations have been characterized to exhibit impaired endothelial function (34, 38). It has previously been suggested that heightened sympathetic nerve activity may contribute to endothelial dysfunction (37); whether the systemic vascular dysfunction is related to elevated MSNA in the Andeans is not clear, but possible (although there are other factors that have been identified as a potential driver of impaired vascular function, such as EE) (35). (34, 38). Poor endothelial functional and/or lowered nitric

oxide (NO) bioavailability may contribute to a differential regulation of blood pressure in this group. However, the absence (or reduction) of a vasodilatory signal to offset transduction would likely result in a larger, rather than smaller, transduction response. In the current study, the Andean group demonstrated a consistently blunted transduction response, suggesting that decreased endothelial function and mechanisms of transduction are acting independently of each other. Further work is needed to elucidate the relationship between vascular function, SNA and neuro-cardiovascular transduction in these populations.

Although some form of sympathetic pathology related to vascular dysfunction may be expected to be more prevalent in Andeans with EE as opposed to non-EE, we did not observe any differences in our initial analysis of transduction (for both MAP and R-R interval) between these groups, indicating that both groups have blunted transduction despite notable hematological differences. While non-EE participants appeared to have elevated burst frequency compared to EE participants, these differences were not statistically significant ($p=0.36$). However, this may be attributable to low sample size within the current data set (participants with EE, n=8; non-EE, n=7). Post hoc power analysis revealed low power for burst frequency (0.3). Based on calculated effect size for the current analysis (0.56), 41 participants would be required in each group to detect significant differences in MSNA burst frequency. Future studies should aim to include a larger cohort in order to specifically characterize pathology and neuro-cardiovascular transduction between EE and non-EE individuals.

CONSIDERATIONS

There are several considerations that should be recognized when interpreting our findings. First, due to differences in basal MSNA, the proportion of single versus multiple bursts was different across groups (Figure 3), resulting in a reduced sample size across burst sequences in groups with lower resting MSNA (specifically for triplet and quad+ sequences). However, a transduction slope was generated for each individual and scaled to the individual's SQ1 (Figure 1), and the

494 generated relationships were linear regardless of dropout in higher (i.e., triplet, quad+) quartiles.
495 Additionally, each individual slope was weighted to account for the number of occurrences for
496 each sequence. Therefore, we believe our data are still representative of transduction across
497 individuals.

498 While we interpreted transduction to be an inverse relationship to resting sympathetic
499 activity, there remains a possibility that a ceiling effect of MSNA exists, in which elevated
500 bursting may not allow for vascular relaxation between bursts or groups of bursts (32). Lack of
501 vascular relaxation between burst sequences could result in pressure remaining elevated
502 following bursts, leading to an interpretation of apparent loss of transduction in groups with
503 higher resting burst incidence (31). However, as previously discussed, vascular adrenergic
504 sensitivity has been shown to be inversely related to resting MSNA (8); this strengthens our
505 interpretation that populations who exhibit higher MSNA have an adaptive reduction in vascular
506 sensitivity to, and thus a true blunting in their neuro-cardiovascular transduction rather than a
507 limitation of our analysis.

508 We recognize that the use of local or total vascular conductance would be highly
509 relevant in characterizing the effect of SNA on cardiovascular tone. However, there are some
510 methodological considerations and limitations in incorporating these measurements. Firstly,
511 there are methodological limitations of collecting continuous vascular flow data during the field
512 studies. We were unable to collect sufficient continuous flow data in all locations or participants
513 to make meaningful comparisons of local vascular conductance. Second, cardiac output
514 variations calculated from ModelFlow have not be validated in the populations or conditions
515 (prolonged hypoxia) of interest. We also recognize that in the current analysis we cannot
516 discern how differences in blood volume, contractility, afterload, stroke volume, and cardiac
517 output may affect the observed transduction (pressor) response. However, previous studies on
518 neuro-cardiovascular transduction have assessed blood pressure as a key cardiovascular
519 outcome using similar methodology to ourselves (14, 15, 32, 41). In these studies, using mean

arterial pressure as an index of vascular tone did not influence the interpretation of differences in neuro-cardiovascular transduction between groups or conditions. Thus, we believe that our analysis of changes in mean arterial pressure (MAP) are still relevant in determining how a given sympathetic stimulus affects cardiovascular function.

Our analysis of changes in R-R interval following burst sequences hinges on the assumption that cardiac sympathetic activity is related to peripheral MSNA. Additionally, acute alterations in heart rate could be interpreted as vagal withdrawal. However, the relationship between burst sequences and the R-R interval response are consistent with what would be expected for increases in sympathetic activity, i.e. R-R interval decreases (heart rate increases) following bursts of sympathetic activity in an apparent dose (increasing sequence length and burst amplitude; Figure 1) dependent manner. Additionally, in attempt to address whether sympathetically mediated effects on heart rate corresponded to concurrent changes in cardiac output, we conducted time to peak analyses in each group for measures of MAP, R-R interval, and finometer derived cardiac output (CO). This analysis revealed that peak changes in BP followed (by ~2-3s) the peak changes in both CO and R-R interval in all groups, while time to peak between CO and R-R interval were similar. This finding suggests that changes in heart rate are associated with concurrent changes in cardiac output. However, the dissociation of the time to peak between heart rate (and cardiac output) and blood pressure also confirms the distinct vascular influence of SNA on the observed BP response.

In this study, the Andean participants were significantly older compared to lowlanders; this could be a contributor to higher prevailing MSNA, as MSNA has been previously reported to be elevated in older populations (40). Additionally, Andean participants had a greater BMI compared to both Sherpa and lowlanders. However, the novel finding of this study is that SNA is inversely correlated with transduction; therefore, although age and/or BMI may account for higher resting SNA, our findings remain relevant in understanding blood pressure control across populations exhibiting sympathetic hyperactivity. Additional ANCOVA analyses revealed that

when both age and BMI were taken into account, burst frequency remained significant (p=0.013 and p=0.034, respectively) as was mean transduction slope (age, p=0.005; BMI, p=0.005).

PERSPECTIVES

In the current study we have demonstrated that sympathetic neuro-cardiovascular transduction is inversely related to resting levels of MSNA. Our data indicates that there is a downregulation (or upregulation) of vascular sensitivity to MSNA based on the prevailing level of signaling. Thus, we suggest that although there are adaptive differences between populations, blood pressure responsiveness to sympathetic outflow is representative of a broader physiological adaptation to maintain control of blood pressure, rather than a consequence specific to hypoxic exposure. Alternatively, MSNA could be increased in order to maintain vascular resistance and therefore blood pressure in cases of blunted vascular responsiveness. Future analysis should incorporate direct adrenergic stimulation or blockade in order to isolate whether this adapted response is mediated by the vasculature (i.e., down-regulation of sensitivity) or neural outflow (i.e., increased MSNA to account for level of sensitivity). Additionally, experimental studies should directly assess the influence of heightened SNA on transduction over time.

AUTHOR CONTRIBUTIONS

LFB and CDS contributed to the conception of the study. GMF and CDS contributed to conception and development of the analytical approach. GMF wrote the analysis software. CKW, MMT, VF, PNA, MS, JPM and CDS contributed to the design and conduct of the experiments carried out as part of the 2016 and 2018 expeditions. LFB, LLS, ERV, SAB, ARS, VLM, JSL, RJF, GAV, CG, MS, JPM and CDS contributed to the acquisition and or analysis of data. LFB, GMF, LLS, ERV, SAB, ARS, VLM, JSL, RJF, GAV, FV, CG, MMT, PNA, MS, JPM, and CDS contributed to the interpretation of data and the writing and critical revision of the manuscript. All authors approved the final version of the manuscript submitted for publication and agree to be accountable for all aspects of the work. All persons included as an author qualify for authorship, and all those who qualify for authorship are listed.

COMPETING INTERESTS

None

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FIGURE CAPTIONS

Figure 1. Example figure of quartile data. Representative bursts for a given quartile (Q1, Q2, Q3, Q4) within each burst sequence type (singlets, couplets, triplets, quad+). Bursts are scaled (LabChart) to mean burst amplitude for each participant. Burst size (i.e., normalized amplitude) and number of bursts (i.e., sequence) increases linearly with the magnitude of the physiological (outcome variable) response. Peak responses (Y axis) to burst sequences were identified within the 15 cardiac cycles (X axis) following a sequence. This quantification of transduction is indicative of how varying levels of neural activity influence the pressor response. This relationship was assessed in each individual participant and then used to obtain a mean transduction slope per group.

Figure 2. Integrated muscle sympathetic nerve activity signal over 30 seconds of baseline. Bursts are scaled (LabChart) to mean burst amplitude for each participant. Individual signal is representative of each group. A: Lowlander at low altitude (344m); B, same lowlander participant at high altitude (5050m); C, Sherpa (5050m); D, Andeans (4380m). Average burst frequency (bursts/min), burst incidence (bursts/100 heart beats), MAP (mmHg), HR (bpm), represented for each individual.

Figure 3. Percentage of total activity within a given sequence (singlet, couplet, triplet, quad+) per group. Data are represented as mean ± SD. A: Lowlanders at low altitude (singlets, n=14; couplets, n=14; triplets, n=8; quad+, n=6). B: Lowlanders at high altitude (singlets, couplets, triplets, n=14; quad+, n=13). C: Sherpa at 5050m (singlets, couplets, triplets, n=8; quad+, n=5). D, Andeans at 4380m (singlets, n=15; couplets, triplets, quad+, n=14). Andeans with diagnosis of excessive erythrocytosis (EE) are depicted by gray circles.

Figure 4. Change mean arterial pressure (MAP; mmHg) following burst sequences (Panel A) and non-burst sequences (Panel B). Data are represented as mean ± SD. Panel A: Lowlanders at low altitude (singlets, couplets, n=14; triplets, n=8; quad+, n=6), Lowlanders at high altitude (singlets, couplets, triplets, n=14; quad+, n=13), Sherpa (singlets, couplets, triplets, n=8; quad+,

n=5), Andeans (singlets, n=15; couplets, triplets, quad+, n=14). Panel B: Lowlanders at low altitude (singlets, couplets, triplets, n=13; quad+, n=14), Lowlanders at high altitude (singlets, couplets, quad+, n=14; triplets, n=13), Sherpa (n=8), Andeans (singlets, n=15; couplets, n=13; triplets, n=11; quad+, n=9). Andeans with diagnosis of excessive erythrocytosis (EE) are depicted by gray circles. Sherpa showed significantly elevated pressor response following burst sequences compared to lowlanders at high altitude ($p=0.010$) and Andeans ($p<0.001$) (Panel A). Lowlanders at high altitude had a significantly greater drop in pressure following non burst sequences compared to at low altitude ($p<0.001$) and compared to Andeans ($p=0.017$) (Panel B).

Figure 5. Change R-R interval (RRI; s) following burst sequences (Panel A) and non-burst sequences (Panel B). Data are represented as mean ± SD. Panel A: Lowlanders at low altitude (singlets, couplets, n=14; triplets, n=8; quad+, n=6), Lowlanders at high altitude (singlets, couplets, triplets, n=14; quad+, n=13), Sherpa (singlets, couplets, triplets, n=8; quad+, n=5), Andeans (singlets, n=15; couplets, triplets, quad+, n=14). Panel B: Lowlanders at low altitude (singlets, couplets, triplet, n=13; quad+, n=14), Lowlanders at high altitude (singlets, couplets, quad+, n=14; triplets, n=13), Sherpa (n=8), Andeans (singlets, n=15; couplets, n=13 triplets, n=11; quad+, n=9). Andeans with diagnosis of excessive erythrocytosis (EE) are depicted by gray circles. Lowlanders and Sherpa had a greater drop in R-R Interval following burst sequences compared to Andeans ($p<0.01$; $p=0.02$) (Panel A).

Figure 6. Mean transduction slope for arterial pressure (MAP; mmHg) plotted against burst frequency (bursts/min). Individual slopes are weighted in order to account for proportions of bursts within each sequence. Panel A, Lowlanders at low (344m) altitude (n=13), r= -0.67; B, Lowlanders at high (5050m) altitude (n=14), r= -0.65; C, Sherpa at high (5050m) altitude (n=8), r=-0.53; D, Andeans at high (4300m) altitude (n=13), r= -0.69. Andeans with diagnosis of excessive erythrocytosis (EE) are depicted by gray circles. Data fitted to linear regression

783 model; 95% confidence interval. Pearson correlation coefficient (r), R squared (r^2) and p value
784 are reported for each group.
785 **Figure 7.** Mean transduction slope for R-R interval (s) plotted against burst frequency. Individual
786 slopes are weighted in order to account for proportions of bursts within each sequence. Panel A,
787 Lowlanders at low (344m) altitude (n=14); B, Lowlanders at high (5050m) altitude (n=14); C,
788 Sherpa at high (5050m) altitude (n=8); D, Andeans at high (4300m) altitude (n=13). Andeans
789 with diagnosis of excessive erythrocytosis (EE) are depicted by gray circles. Data fitted to
790 exponential decay function for regression.
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Table 1: Participant demographics and physiological characteristics

	LOW ALTITUDE NATIVES		HIGH ALTITUDE NATIVES			
	Low Altitude (344m)	**High Altitude (5050m)**	**Tibetan Sherpa (5050m)**	**Andean (4380m)**	**Main Effect of Group**	**Paired Ttest (Low to High)**
	(n= 14; 12 M)	**(n= 14; 12 M)**	**(n= 8; 8 M)**	**(n= 15; 15 M)**	**p value**	**p value**
Age (yrs)	27 ± 1	27 ± 1	32 ± 5	42 ± 3	**0.001**	-
Height (cm)	177 ± 2	177 ± 2	168 ± 3	161 ± 1	**< 0.001**	-
Weight (kg)	72 ± 3	69 ± 2	64 ± 4	70 ± 3	0.396	-
BMI (kg/m²)	23 ± 1	22 ± 1	23 ± 1	27 ± 1	**0.002**	-
Heart Rate (bpm)	53 ± 3	64 ± 4	68 ± 5	68 ± 3	0.625	**0.03**
R-R Interval (sec)	1.2 ± 0.10	0.97 ± 0.05	0.93 ± 0.11	0.9 ± 0.04	0.655	**0.025**
Mean Arterial Pressure (mmHg)	84 ± 2	86 ± 3	83 ± 3	86 ± 2	0.773	0.638
MAP Delta Mean (mmHg)	1.1 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	0.87 ± 0.1	**0.009**	0.26
Systolic Arterial Pressure (mmHg)	118 ± 3	112 ± 3	110 ± 3	110 ± 2	0.871	0.072
Diastolic Arterial Pressure (mmHg)	67 ± 2	70 ± 3	65 ± 3	72 ± 1	0.154	0.146
Cardiac Output (L/min)	5.3 ± 0.3	5.3 ± 0.3	6 ± 0.6	6 ± 0.3	0.441	1
Total Peripheral Resistance (mmHg/L/min)	17 ± 1	17 ± 1	16 ± 2	15 ± 1	0.463	0.595
Total Peripheral Conductance (L/mmHg/min)	0.063 ± 0.003	0.063 ± 0.003	0.07 ± 0.01	0.068 ± 0.004	0.218	0.612
Peripheral Oxygen Saturation (%)	-	82 ± 1	82 ± 1	81 ± 1	0.595	-
Burst Incidence (bursts/100 heart beats)	22 ± 3	47 ± 4	30 ± 5	57 ± 4	**0.002**	**< 0.001**
Burst Frequency (bursts/min)	11 ± 1	30 ± 2	23 ± 4	39 ± 3	**0.006**	**< 0.001**

BMI, Body Mass Index; Cardiac output, total peripheral resistance and total peripheral conductance calculated from finger photoplethysmography. One-way ANOVA used to determine differences between high altitude groups; paired, two tailed t-tests used to compare lowlanders from low to high altitude. Values are mean +/- SE.